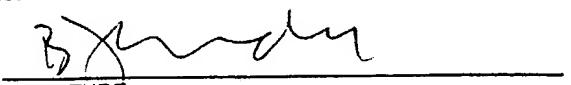


FORM PTO-1390 (REV 11-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER 3557-12
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) <b>10/088025</b>	
INTERNATIONAL APPLICATION NO. <b>PCT/EP00/07625</b>	INTERNATIONAL FILING DATE <b>5 August 2000</b>	PRIORITY DATE CLAIMED <b>15 September 1999</b>	
TITLE OF INVENTION <b>PLANTS WITH A MODIFIED AMINO ACID CONTENT AND THEIR GENERATION</b>			
APPLICANT(S) FOR DO/EO/US <b>REINDL et al</b>			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.</li> <li>4. <input checked="" type="checkbox"/> The U.S. has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).             <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is attached hereto.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</li> </ol> </li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has <b>NOT</b> expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li>10. <input type="checkbox"/> A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol>			
Items 11 To 20 below concern document(s) or information included:			
<ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li>14. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>15. <input type="checkbox"/> A substitute specification.</li> <li>16. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.</li> <li>18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>20. <input checked="" type="checkbox"/> Other items or information. <u>PTO-1449 and copy of International Search Report</u></li> </ol>			

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.45) Unknown		INTERNATIONAL APPLICATION NO. PCT/EP00/07625	ATTORNEY'S DOCKET NUMBER 3557-12		
21. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS - PTO USE ONLY	
<b>BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):</b> <ul style="list-style-type: none"> <li>-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$1040.00</li> <li>-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$890.00</li> <li>-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO ..... \$740.00</li> <li>-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$710.00</li> <li>-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00</li> </ul>					
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				\$ 890.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).				\$ 0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	19	-20 = 0	X	\$18.00	\$ 0.00
Independent Claims	1	-3 = 0	X	\$84.00	0.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)				\$280.00	\$ 280.00
				<b>TOTAL OF ABOVE CALCULATIONS =</b>	<b>\$ 1170.00</b>
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.					0.00
				<b>SUBTOTAL =</b>	<b>\$ 1170.00</b>
Processing fee of \$130.00, for furnishing the English Translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)).					0.00
				<b>TOTAL NATIONAL FEE =</b>	<b>\$ 1170.00</b>
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). \$40.00 per property				+	\$ 0.00
Fee for Petition to Revive Unintentionally Abandoned Application (\$1280.00 - Small Entity = \$640.00)				\$ 0.00	
				<b>TOTAL FEES ENCLOSED =</b>	<b>\$ 1170.00</b>
				Amount to be refunded	\$
				Charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$1170.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 14-1140 in the amount of \$ _____ to cover the above fees. A duplicate copy of this form is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A <u>duplicate</u> copy of this form is enclosed. d. <input checked="" type="checkbox"/> The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.					
<b>NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
<b>SEND ALL CORRESPONDENCE TO:</b>  <b>B. J. Sadoff</b> NAME					
NIXON & VANDERHYE P.C. 1100 North Glebe Road, 8 <sup>th</sup> Floor Arlington, Virginia 22201-4714 Telephone: (703) 816-4000					
<b>36,663</b> <b>March 14, 2002</b> REGISTRATION NUMBER Date					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#57

In re Patent Application of

REINDL et al

Atty. Ref.: 3557-12

Serial No. 10/088,025

Group:

Filed: March 14, 2002

Examiner:

For: PLANTS WITH A MODIFIED AMINO ACID CONTENT  
AND THEIR GENERATION

\* \* \* \* \*

September 13, 2002

Honorable Assistant Commissioner of Patents  
Washington, DC 20231

Sir:

AMENDMENT

Responsive to the Notification dated May 15, 2002, entry and consideration of the following amendments and remarks are requested; the period for response having been extended up to and including September 15, 2002, by submission of the requisite petition and fee, attached.

IN THE SPECIFICATION:

Amend the specification as follows.

Delete the paragraph spanning page 5, lines 29-33 and insert the following  
therefor:

--The invention furthermore relates to an ATP/ADP translocator gene for use in one of the above-described plants with an *Arabidopsis thaliana* amino acid sequence (EMBL Accession No. Z49227) encoding by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).--

**In re Application of: REINDL et al**  
Serial No. 10/088,025

Insert the attached revised Figures 1 and 2 in place of the similarly-numbered, originally-filed Figures.

Please insert the attached Sequence Listing after the claims pages.

**IN THE CLAIMS:**

Amend the claims as follows:

6. (Twice Amended) ATP/ADP translocator gene for use in a plant according to Claim 1 with an *Arabidopsis thaliana* amino acid sequence (EMBL Accession No. Z49227) encoded by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).

**REMARKS**

Reconsideration is requested.

Responsive to the Notification dated May 15, 2002, a copy of the requisite Declaration is attached.

A copy of the Notification dated May 15, 2002 is attached.

The specification has been amended to include the attached revised Figures. A marked-up copy of the amended Figures is attached wherein changes are shown as underlined text.

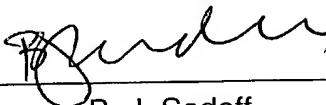
The specification has been amended to include the attached Sequence Listing. The specification has been amended to include sequence identifiers, consistent with the attached Sequence Listing. The attached paper and computer readable copies of the Sequence Listing are the same. No new matter has been added. A separate Statement to this effect is attached.

An early and favorable Action on the merits is requested.

In re Application of: REINDL et al  
Serial No. 10/088,025

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

By: 

B. J. Sadoff

Reg. No. 36,663

1100 North Glebe Road, 8th Floor  
Arlington, VA 22201-4714  
Telephone: (703) 816-4000  
Facsimile: (703) 816-4100

In re Application of: REINDL et al  
Serial No. 10/088,025

**MARKED-UP COPY OF AMENDED SPECIFICAITON AND CLAIMS**

**IN THE SPECIFICATION:**

Amend the specification as follows.

Delete the paragraph spanning page 5, lines 29-33 and insert the following therefor:

--The invention furthermore relates to an ATP/ADP translocator gene for use in one of the above-described plants with an *Arabidopsis thaliana* [nucleotide] amino acid sequence (EMBL Accession No. Z49227) encoding [the amino acid] by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).--

**IN THE CLAIMS:**

Amend the claims as follows:

6. (Twice Amended) ATP/ADP translocator gene for use in a plant according to Claim 1 with an *Arabidopsis thaliana* [nucleotide sequence] amino acid sequence (EMBL Accession No. Z49227) [encoding the amino acid] encoded by the nucleotide sequence shown in Fig. 1 (SEQ ID NO:1).

Fig. 1: *Arabidopsis thaliana* cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Z49227) (SEQ ID NO:1)

Fig. 2: Solanum tuberosum cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Y10821) (SEQ ID NO:2)

atggaaagggtttacaacaagaggcctttgccttctaaacccaaaatcaaggctttaccat  
tgccctcaaggggcttaaggaacagattcaattcttaagtgtttaaagcctaattcttaatggggtt  
cttatacttcaatgggtcaaaaagtcaaggcttgcacacaagcctcagttttggccaaaagaag  
agggtttccaatatgcaaagctgaggctgctgctgctggcagctgatggacagccacttttgtt  
gaaaaggagcaacctaagttatgggattgaacttgtgacccttaagaaaattataccactggggcg  
atgtctttgtattctgttaattatacaatccttagggatactaaggatgttgttaacagctaaaggg  
tccagtgctgagattatcccttctgaaaacttgggtgaattgcctatggctattggattcatgctttgtac  
acaaaagtggctaigtgtgtcaaaggaggcttttatactgttatacttcctttattgcattcttgggc  
gttgggtttgtttgtatcctcttagcaattacttcaccctacagctttgctgataagctctcaataccctgg  
tccaagattctggaccaattgctattctgaggatctggagttctgcttgtctatgtcatggctgagcttg  
gggaagtgtgggtttcagttactcttgggatttgctaatcagatcacgactgtcgatgaggctaaaga  
gattctatccttttgttggacttggagcgaatgtgtcttatttctctggcgcacagtgaagtacttttag  
cttggagaagcttttaggtcctggagttgtgttggctatccctgaaaggaatgtgtctcccaactcgtagcaa  
gaagaagaaggtaaaacctaacatgaccacaatggagagcttgaagtcttggctttcaaaaatatat  
cagggatctgccacattgggttagcatatggcattgtatcaaccttggtaagttacatggtaactcaa  
agctcaaagctcagttccaaagccccatgaataactccatcatgggtacttcaactgtactgg  
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aagataacacctacagtctgtccctaccggagttggttctccctgctttgttggggcacctctagc  
acctacttgcgaagttggaaatgactcccttagcagctgtctatgtgggtgaatgcagaacatttc  
agtaagagtgc当地atgtttggatggccctgcaaagaaatggctacattccttggatgaggaca  
ccaaggtaaagggaaggcagcaatcgatgtgtcaatccactggaaatctggaggagcttg  
atacaacagttcatgatttgactttggttcacttgccagctgacaccctaccttggcgggtgtcttagt  
aattgttctgc当地gggtggagcagccaaatgtttggatggacagttcactcaattacgccaagaagaa  
gatcttggagaaggaaatggagagagcatcgtaagatccctgtgtctcaaaaatgaaaatggaa  
atggccctctctcaagtgtcatcactaaatcccgctggagggtacttaccaacgcctcatcggaacc  
ctccctcccaaggagcctgtaa

# Marked-Up Copy of Amended Figures

Fig. 1: Arabidopsis thaliana cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Z49227) (SEQ ID NO:1)

atgaaagctgtgattcaaaccagaggcgtctcttacccaccaaaccatggagtgagaagcca  
acttcagcctccatggctaaagcagagactttcgccgcgaagccaagaatctacatgggtgtct  
ctatccttaacgggcacaagaaattcaaacccttgagccaaccctgcattggatttcgattcccaca  
aagagagaagcaccgagttcatatgcaggcgaggcgccgtgtggcagggagctgtctcg  
gcgaagcgattccgcagctgttagccctcgccaaagatttcggtgtggagggttgcacacccatgg  
gattatcccttaggattgtttttgtattttcaattacacaattctgagggataaaaggatgtcttg  
gtgggtacggcgaaaggaagttctgtctgagattataccttctgaagacttgggtgaatctccatggc  
cattgggttatgctcctctacactaaactctccaatgttctccaagaaggctgtttacactgttattgtc  
cctttcatcatctactttggggcttggttcgcatgttccatgcactatattcaccggaaagctct  
cgcagataagctcctacaaccctcgcccaagattcatgggtctattgcaatattgcggattggagtt  
tctgtttttatgtttaggttagctgttt  
atcacaactgtggatgaagccaagaaattctatcccttgcggcattggagccaatgttgcactgtttt  
tcaggaagaaccgtgaaatactctctaacttgagaaagaatctggctggagttgacggcagttcg  
ttgaaagccatgtgagcattgtggatggactcgcatgtctctattgggtggcatacggtattatca  
tatgttcccttccaacccttagcaagaacaagaaggagaaaccgaagatgggaacgatggaaag  
cttgaagttcttggatcatcaccatacttagagatctgtacttttttttttttttttttttttttt  
atcttggaagtccatggaaatcaaagctaaagctcagttccctagcccaatgatgcitctcagccaatct  
tatgggagcattctcaacctgcacgggttgcaacattcacaatgtatgcitctcagccaatct  
ataagtatgggtggggagtagtgcacccatgggttttttttttttttttttttttttttttttt  
ctctaaatattgttggcggccattcgaccactgttgcacccatgggtatgacaccgtactgcagctgt  
gtatgtcggtgccttcagaatatctcagcaagatgcacccatgggttttttttttttttttttt  
atggcctatccatggatgaggacaccaaggtaaaggcaagatgcgttttttttttttttttttt  
cccataggaaaatcagggggagcttaatacagcagttcatgtatcccttggatcactgcgaaatt  
caacccgtatcttagaatgatctgttttttttttttttttttttttttttttttttttttttt  
ggacagtcaacacagcttgcgtctgaagaagagcttgagaaggaaatggagagagcttcatcggtga

# Marked-Up Copy of Amended Figures

Fig. 2: Solanum tuberosum cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Y10821) (SEQ\_ID NO:2)

REINDL et al  
Serial No. 10/088,025  
Filed: March 14, 2002

Atty. Ref.: 3557-12

Group:

Examiner:

For: PLANTS WITH A MODIFIED AMINO ACID CONTENT  
AND THEIR GENERATION

**September 13, 2002**

Honorable Assistant Commissioner of Patents  
Washington, DC 20231

Sir:

## **STATEMENT**

The attached paper and computer readable copies of the Sequence Listing are the same. No new matter has been added.

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

By:

B. J. Sadoff  
Reg. No. 36,663

1100 North Glebe Road, 8th Floor  
Arlington, VA 22201-4714  
Telephone: (703) 816-4000  
Facsimile: (703) 816-4100

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

**REINDL et al**

Atty. Ref.: 3557-12

Serial No. **Unknown**

Group:

National Phase of: **PCT/EP00/07625**International Filing Date: **5 August 2000**Filed: **March 14, 2002** Examiner:For: **PLANTS WITH A MODIFIED AMINO ACID CONTENT  
AND THEIR GENERATION****March 14, 2002**Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

**PRELIMINARY AMENDMENT**

Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend as follows:

**IN THE SPECIFICATION**

Page 1, after the title insert the following:

-- This application is the US national phase of international application

PCT/EP00/07625 filed August 5, 2000 which designated the U.S. --.

**IN THE CLAIMS**

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

4. (Amended) Transformed plant and its progeny according to claim 1, characterized in that it exhibits one or more essential amino acid(s) whose content is increased over that of the untransformed plant.

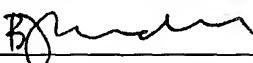
5. (Amended) Transformed plant and its progeny according to claim 1, characterized in that it is a useful plant.
6. (Amended) ATP/ADP translocator gene for use in a plant according to claim 1 with an *Arabidopsis thaliana* nucleotide sequence (EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.
9. (Amended) ATP/ADP translocator gene according to claim 6 with an upstream, operably linked promoter.
10. (Amended) Gene structure comprising an ATP/ADP translocator gene according to claim 6 and regulatory sequences linked operably to this gene.
11. (Amended) Vector comprising an ATP/ADP translocator gene according to claim 6.
13. (Amended) Seeds of the plant according to claim 1.
14. Tissue or cells or material capable of propagation from the plant according to claim 1.
15. (Amended) Method of generating a plant with an increased amino acid content, characterized in that an ATP/ADP translocator gene according to claim 6 is transferred by recombinant methods.
16. (Amended) Use of the transformed plant according to claim 1 as useful plant or fodder plant.
17. (Amended) Use of the transformed plants according to claim 1 or of tissue or cells thereof or of extracts thereof in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

REINDL et al  
Serial No. Unknown

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,  
NIXON & VANDERHYE P.C.  
By:   
B. J. Sadoff  
Reg. No. 36,663

BJS:Imy  
1100 North Glebe Road, 8th Floor  
Arlington, VA 22201-4714  
Telephone: (703) 816-4000  
Facsimile: (703) 816-4100

VERSION WITH MARKINGS TO SHOW CHANGES MADE

4. (Amended) Transformed plant and its progeny according to [one of Claims 1 to 3] claim 1, characterized in that it exhibits one or more essential amino acid(s) whose content is increased over that of the untransformed plant.

5. (Amended) Transformed plant and its progeny according to [one of Claims 1 to 4] claim 1, characterized in that it is a useful plant.

6. (Amended) ATP/ADP translocator gene for use in a plant according to [one of Claims 1 to 5] claim 1 with an *Arabidopsis thaliana* nucleotide sequence(EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.

9. (Amended) ATP/ADP translocator gene according to [one of Claims 6 to 8] claim 6 with an upstream, operably linked promoter.

10. (Amended) Gene structure comprising an ATP/ADP translocator gene according to [one of Claims 6 to 9] claim 6 and regulatory sequences linked operably to this gene.

11. (Amended) Vector comprising an ATP/ADP translocator gene according to [one of Claims 6 to 9 or a gene structure according to Claim 10] claim 6.

13. (Amended) Seeds of the plant according to [one of Claims 1 to 5] claim 1.

14. Tissue or cells or material capable of propagation from the plant according to [one of Claims 1 to 5] claim 1.

15. (Amended) Method of generating a plant with an increased amino acid content [according to one of Claims 1 to 5], characterized in that an ATP/ADP translocator gene according to [one of Claims 6 to 9 or a gene structure according to

REINDL et al  
Serial No. Unknown

Claim 10 or a vector according to Claim 11 or 12] claim 6 is transferred by recombinant methods.

16. (Amended) Use of the transformed plant according to [one of Claims 1 to 5] claim 1 as useful plant or fodder plant.

17. (Amended) Use of the transformed plants according to [one of Claims 1 to 5] claim 1 or of tissue or cells thereof or of extracts thereof in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

WO 01/20009

4/PDV

PCT/EP00/07625

Plants with a modified amino acid content  
and their generation

The present invention relates to transformed plants and  
5 their progeny whose regulatory sequences and/or gene  
copy number of the ATP/ADP translocator gene are  
modified in such a way that they exhibit one or more  
amino acids simultaneously in modified amounts in  
comparison with an untransformed plant. The present  
10 invention furthermore relates to a method of generating  
these plants and to their use as useful plant or in  
sectors of the feedstuff industry.

Humans and animals are only capable of synthesizing 11  
15 out of the 20 amino acids and therefore depend on  
taking up the 9 what are known as essential amino acids  
via the food. The nutrition of humans and livestock is  
predominantly based on plant components. The essential  
amino acids include lysine, tryptophan, valine,  
20 leucine, isoleucine, methionine, threonine, phenyl-  
alanine and histidine.

The fact that the concentration of these amino acids in  
food plants is frequently only very low gives rise to a  
25 problem. This is why grain mixtures and vegetable-based  
foodstuffs are frequently supplemented with syntheti-  
cally produced amino acids in order to increase their  
nutritional value.

30 In the past, a number of avenues were followed to  
increase the amounts of free amino acids, i.e. amino  
acids which are not found in proteins. However, these  
attempts focused mainly on traditional breeding and on  
the selection of mutants.

35

In the recent past, there have been increasing attempts  
to increase the amounts of essential amino acids by  
applying molecular-genetic techniques. WO 97/28247,

WO 98/13506 and WO 97/35023 describe first attempts at extending the heterologous expression of a seed-specific storage protein which is high in lysine or methionine. The disadvantage here is that the amino acids are stored in proteins, that is to say that, again, the increase takes the form of an increase in bound amino acids.

Furthermore, a large number of attempts for directly controlling amino acid biosynthesis are known. In these attempts, individual genes encoding specific amino acid biosynthesis enzymes were overexpressed in plants, resulting in an increase in the biosynthesis end products in question.

As an alternative, it has furthermore been attempted to control the reaction kinetics of enzymes. What is known as the product inhibition of enzymes causes a particular problem here. For example, Shaul and Galili (1993; Plant Mol Biol 23: 759-768) and Falco et al. (1995; Bio/Technology 13: 577-582) describe plants which overproduce free lysine, coupled with a decrease in free threonine. The enzyme responsible is aspartate kinase, the first enzyme in the biosynthesis of the amino acids derived from aspartate and which is inhibited allosterically by lysine. To circumvent this feedback inhibition, recombinantly modified aspartate kinase genes were overexpressed in plants (WO 94/25605). This modified aspartate kinase has a greatly reduced feedback inhibition by lysine and threonine, leading to an increase in lysine. This aspartate kinase which is insensitive to feedback inhibition by lysine was furthermore overexpressed together with other biosynthesis enzymes. Suitable experiments were carried out in *Corynebacteria* (1991, Applied and Environmental Microbiology 57: 1746-1752). In these bacteria, however, not only an increase in lysine results, but also a pronounced decrease in the growth rate, which, in turn, has a negative effect on

the lysine balance.

Experiments with plants having both a feedback-insensitive aspartate kinase and a feedback-insensitive dihydropicolinate synthase are described by Shaul and Galili (1993; Plant Mol Biol 23: 759-768). These two enzymes have a key position in amino acid biosynthesis. However, overexpression of these bottleneck enzymes did not result in the hoped-for increase of the two amino acids lysine and threonine. Rather, only the free lysine content was increased, with the free threonine content simultaneously going down drastically.

Beyond the overexpression of one or two amino acid biosynthesis genes, WO 98/56935, EP 0 854 189 and EP 0 485 970 describe multi-gene approaches which aim at influencing the amounts of one or more amino acids simultaneously in one plant. A prerequisite therefor is the genetic modification of a plant with regard to several genes; i.e. it would be necessary to generate a multi-transgenic plant. However, these methods are very complicated. Moreover, such massive interference with hereditary material of the plant increasingly harbours risks of unpredictable side reactions.

It is an object of the present invention to provide transgenic plants and a method of generating them without the abovementioned disadvantages.

We have found that this object is surprisingly achieved in accordance with the invention by providing a transformed plant whose regulatory sequences and/or gene copy number of an ATP/ADP translocator gene are modified in such a way that it exhibits one or more amino acids simultaneously in modified amounts in comparison with a corresponding untransformed plant.

The transformed plants are distinguished in accordance with the invention by exhibiting predominantly one or more essential amino acid(s) in modified amounts.

In particular, the plants according to the invention exhibit one or more essential amino acid(s) whose content is increased over that of the untransformed 5 plants.

The transformed plants are, in accordance with the invention, useful plants, preferably economically relevant plants, such as, for example, potatoes or 10 maize. However, the present invention is not restricted to these genera.

The present invention relates both to the above-mentioned transformed plants, their seeds and progeny 15 and also to tissue, cells or material capable of propagation derived from these transformed plants.

In one embodiment of the present invention in which the gene encoding the ATP/ADP translocator is overexpressed 20 in accordance with the invention in potatoes, an increase in amino acids which are interesting from the nutritional and economical point of view, such as lysine, methionine, threonine, valine, tryptophan, histidine, isoleucine and leucine, is achieved.

25 In the transformed plant referred to as line 98, the amount of free lysine is increased by 28%; in the transgenic plant referred to as line 62, the increase in the amount of free lysine is 25.75%. Surprisingly, 30 an at least 25% increase in the lysine content is achieved by increasing the ATP/ADP translocator activity in the plants by only 50%. Furthermore, the amount of methionine in line 98 is increased by 11%. In addition to increased amounts of lysine and methionine, 35 increased amounts of the essential amino acids valine (12% in line 98), tryptophan (50% in line 98), threonine (12.5% in line 98), histidine (23.5% in line 98 and 20% in line 62), isoleucine (25% in line 98) and leucine (40% in line 98) are also found.

Accordingly, overexpression of the ATP/ADP translocator in antisense orientation results in a reduction of the amounts of amino acid in the respective transformed 5 plants, referred to as lines 594 and 595. In the case of lysine, only approximately a quarter of the wild-type lysine quantity is found here; while in the case of methionine only approximately not more than one eighth of the wild-type methionine quantity is found.

10 An overview over the amino acid spectrum in the wild type of potato, *Solanum tuberosum*, and in transformed potato plants is compiled in Table 1. In this embodiment of the invention, the total amount of free amino 15 acids in the transformed potato plants is increased by approximately 7% over the wild type.

20 A particular advantage of the present invention is that the increased expression of a single gene, viz. of the ATP/ADP translocator, can bring about a specific increase of several, predominantly essential, amino acids simultaneously.

25 In accordance with the invention, the transformed plant is distinguished by the fact that it has an increased transport capacity for ATP into the chloroplast membrane.

30 The invention furthermore relates to an ATP/ADP translocator gene for use in one of the above-described plants with an *Arabidopsis thaliana* nucleotide sequence (EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.

35 In accordance with the invention, the use of any ATP/ADP translocator gene from organisms which have chloroplasts is feasible. Preferred organisms are plants in general, green algae or mosses.

Normally, the ATP/ADP translocator gene is localized in the internal chloroplast membrane, where it is responsible for the antiport, i.e. the opposite transport, of ATP and ADP, by exporting chloroplast ADP into the cytosol in exchange for ATP. Owing to the increased activity of this ATP/ADP translocator, the amount of ATP in the chloroplast is increased (Neuhaus et al., 1997, The Plant Journal 11: 73-82). Tjaden et al. (1998, Plant Journal 16: 531-540) demonstrated that the uptake of ATP into potato chloroplasts owing to overexpression of the ATP/ADP translocator out performs the uptake capacity of the wild type by an average of 50%. These energy-rich ATP molecules, which are now increasingly available, can be exploited for the increased biosynthesis of starch and fatty acids as described by Möhlmann et al., 1994, Planta, 194: 492-497; Neuhaus et al., 1993, Plant Physiology 101: 573-578; Tjaden et al., 1998, Plant Journal 16: 531-540.

20

In accordance with the invention, an ATP/ADP translocator gene with a naturally, chemically synthesized, modified, artificially generated nucleotide sequence with essentially the same action or with heterologous nucleotide sequences encoding an ATP/ADP translocator or allelic variations or isoforms thereof or with mixtures thereof may also be employed.

30

Sequences which encode an ATP/ADP translocator gene with essentially the same action are those sequences which, despite a deviating nucleotide sequence, retain the desired functions. Equivalents with the same action thus encompass naturally occurring variants of the described sequences, but also artificial nucleotide sequences, for example those obtained by chemical synthesis, which are adapted to the codon usage of a plant.

A nucleotide sequence with the same action is also

understood as meaning, in particular, natural or artificial mutations of an originally isolated sequence encoding an ATP/ADP translocator and retaining the desired function. Mutations encompass substitutions, 5 additions, deletions, exchanges or insertions of one or more nucleotide residues. Thus, for example, the present invention also extends to those nucleotide sequences which are obtained by modifying the ATP/ADP translocator nucleotide sequence. The purpose of such a 10 modification may be, for example, the further delimitation of the coding sequence contained therein, or else, for example, the insertion of further cleavage sites for restriction enzymes.

15 Nucleotide sequences with the same action are also those variants whose function is reduced or increased compared with the original gene or gene fragment.

Suitable DNA sequences are, furthermore, artificial DNA 20 sequences as long as they confer the desired properties, as described above. Such artificial DNA sequences can be determined for example by back-translating proteins constructed by means of molecular modelling which have an ATP/ADP translocator activity, or else by 25 in-vitro selection. Especially suitable are coding DNA sequences which have been obtained by back-translating a polypeptide sequence in accordance with the host-plant-specific codon usage. The specific codon usage can be determined readily by a skilled worker familiar 30 with plant genetic methods by means of computer evaluations of other, known genes of the plant to be transformed.

The invention furthermore encompasses an ATP/ADP translocator gene which is operably linked to regulatory 35 nucleotide sequences. The regulatory sequences also include, inter alia, an upstream promoter which makes possible expression in plants.

Operable linkage is understood as meaning the sequential arrangement of, for example, promoter, coding sequence, terminator and, if appropriate, further regulatory elements in such a way that each of the 5 regulatory elements can fulfil its intended function upon expression of the coding sequence. Suitable as promoter is, in principle, any promoter capable of governing the expression of foreign genes in plants. A plant promoter or a promoter derived from a plant virus 10 is preferably used. Particularly preferred is the cauliflower mosaic virus CaMV 35S promoter (Franck et al., Cell 21 (1980), 285-294). As is known, this promoter contains various recognition sequences for transcriptional effectors which, in their totality, 15 bring about permanent and constitutive expression of the gene introduced (Benfey et al., EMBO J, 8 (1989), 2195-2202).

Further sequences which are preferred for operable linkage, but not limited thereto, are transcription 20 terminators and translation enhancers, such as the tobacco mosaic virus 5' leader sequence (Gallie et al., Nucl. Acids Res. 15 (1987), 8693-8711).

Adaptors or linkers can be attached to the fragments to 25 connect the DNA fragments with each other. Preferably, the promoter and the terminator regions can be provided, in the direction of transcription, with a linker or polylinker comprising one or more restriction sites for insertion of this sequence. As a rule, the linker 30 has 1 to 10, preferably 1 to 8, especially preferably 2 to 6, restriction sites. In general, the linker has a size of less than 100 bp within the regulatory regions, frequently less than 60 bp, but at least 5 bp. The promoter can be native, or homologous, or else foreign, 35 or heterologous, relative to the host plant.

The invention furthermore relates to a gene structure comprising an ATP/ADP translocator gene and regulatory sequences linked operably to this gene and a vector

comprising an ATP/ADP translocator gene or a gene structure as described above. In this context, the vector may comprise additional regulatory nucleotide sequences, preferably from the group of the promoters, 5 terminators or translation enhancers, and nucleotide sequences for the replication in a suitable host cell or for integration into its genome.

Using recombination and cloning techniques known per se, the gene structures can be cloned into suitable 10 vectors which make possible their amplification in host cells such as, for example, plants, plant tissues or plant cells. Suitable vectors are described, inter alia, in "Methods in Plant Molecular Biology and 15 Biotechnology" (CRC Press), Chapter 6/7, pp. 71-119 (1993).

Suitable as cloning vectors for *E. coli* as host cell are, in particular, pBR322, pUC series, M13mp series 20 and pACYC184. Especially preferred are binary vectors which are capable of replication both in *E. coli* and in, for example, agrobacteria. An example of a binary vector which may be mentioned is pBIN19 (Bevan et al., Nucl. Acids Res. 12 (1984), 8711). For example, the 25 gene structure according to the invention may also be incorporated into tobacco transformation vector pBIN-AR-TP.

The present invention furthermore relates to a method 30 of generating an above-described transformed plant, wherein an ATP/ADP translocator gene, a gene structure or a vector of the above-described type is transferred into the plant or tissue or cells thereof by recombinant methods. In general, the transfer of DNA is to be 35 understood as meaning the transformation of plants, plant tissue or plant cells.

Suitable methods for the transformation and regeneration of plants from plant tissues or plant cells for

transient or stable transformation are protoplast transformation by polyethylene glycol-induced DNA uptake, the biolistic method with the gene gun - what is known as the particle bombardment method -, electro-  
5 poration, the incubation of dry embryos in DNA-containing solution, microinjection and the agrobacterium-mediated gene transfer. The methods mentioned are described, for example, in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic  
10 Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993), 128143 and in Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205225).

15 The present invention thus makes possible the generation of economically valuable useful plants which are distinguished by a substantially increased amino acid content, in particular a substantially increased essential amino acid content.

20 The present invention furthermore relates to the use of the transformed plant as useful plant or fodder plant. Since the content of several essential amino acids may be increased in the useful plants according to the  
25 invention, in particular simultaneously, expensive supplementation of the feedstuffs with amino acids which previously had to be produced or obtained separately by conventional methods and externally admixed to the feed can advantageously be dispensed with.

30

The transformed plant in accordance with the invention, its seed and its progeny, and tissue or cells thereof or extracts thereof, can furthermore be used in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

In the following text, the present invention is illustrated in greater detail by use examples which,

however, do not limit the scope of the invention:

1. General cloning methods

Cloning methods such as, for example, restriction  
5 cleavages, agarose gel electrophoresis, purification of  
DNA fragments, transfer of nucleic acids to nitro-  
cellulose and nylon membranes, linking DNA fragments,  
transformation of *E. coli* cells, bacterial cultures,  
phage multiplication, and sequence analysis of recom-  
10 binant DNA, were carried out as described by Sambrook  
et al. (1989, Cold Spring Harbor Laboratory Press:  
ISBN 0-87969-309-6).

The bacterial strains used (*E. coli*, XL-I Blue) were  
15 obtained from Stratagene (Heidelberg) or Qiagen  
(Hilden). The agrobacterial strain used for the trans-  
formation of the plants (*Agrobacterium tumefaciens*,  
C58C1 with the plasmid pGV2260 or pGV3850kan) was  
described by Deblaere et al. in Nucl. Acids Res. 13  
20 (1985), 4777. As an alternative, the agrobacterium  
strain LBA4404 (Clontech) or other suitable strains may  
also be employed.

The vectors pUC19 (Yanish-Perron, Gene 33 (1985),  
25 103-119) pBluescript SK- (Stratagene), pGEM-T (Promega),  
pZero (Invitrogen) pBin19 (Bevan et al., Nucl. Acids  
Res. 12 (1984), 8711-8720) and pBinAR (Höfgen and  
Willmitzer, Plant Science 66 (1990), 221-230) may be  
used for cloning.

30

2. Transformation of agrobacteria

*Agrobacterium tumefaciens* was transformed following the  
method of Höfgen and Willmitzer (Nucl. Acid Res. (1988)  
16, 9877). The agrobacteria were grown in YEB medium  
35 (Vervliet et al., J. Gen. Virol. (1975) 26, 33).

3. Sequence analysis of recombinant DNA

Recombinant DNA molecules were sequenced using a Licor  
laser fluorescence DNA sequencer (sold by MWG Biotech.,

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Ebersbach) following the method of Sanger (Sanger et al., Proc. Natl. Acad. Sci. USA 74 (1977), 5463-5467).

4. Construction of a plant transformation vector with AATP1 in sense orientation

To construct a vector for transforming plants, a 2230 bp EcoRV/BamHI fragment of the *Arabidopsis thaliana* AATP1 cDNA (the cloning of AATP1 from *Arabidopsis thaliana* is described in Kampfenkel et al., 10 FEBS Letters 374 (1995), 351-355 and Neuhaus et al., The Plant Journal 11: 73-82) is ligated into an SmaI/EcoRV- and BamHI-cut vector pBinAR (Höfgen and Willmitzer, Plant Science 66 (1990), 223-230). Insertion of the cDNA fragment gives rise to a gene construct comprising the cauliflower mosaic virus 35S promoter (540 bp) and the protein-encoding region of the *Arabidopsis thaliana* ADP/ATP translocator 1 (AATP1). The cDNA fragment is fused in sense orientation to the 35S promoter in pBinAR. The polyadenylation signal of the *Agrobacterium tumefaciens* octopine synthase gene (215 bp) follows in the 3' direction of the inserted AATP1 fragment.

The overall size of the plasmid pBIN AR-AATP1 (Fig. 3) is approx. 14.2 kb.

5. Insertion of the plasmid pBINAR-ATTP1 into the genome of potato plants

The plasmid is transferred into potato plants with the aid of *Agrobacterium tumefaciens* as described by 30 Rocha-Sosa et al. (EMBO J. 8 (1989), 23-29). Transgenic potato plants with an increased level of the plastid ADP/ATP translocator 1 mRNA acted as positive control for the transformation. Detection is by Northern blot analysis. To this end, RNA is isolated from potato leaf 35 and tuber tissue following standard protocols. 50 µg of RNA are separated on an agarose gel (1.5% agarose, 1 × MEN buffer, 16.6% formaldehyde). Following electrophoresis, the RNA is transferred to a Hybond N nylon membrane (Amersham, UK) by capillary blotting, using

20 x SSC. The RNA is immobilized on the membrane by UV  
irradiation, and the membrane is prehybridized for  
2 hours in phosphate hybridization buffer (Sambrook et  
al., 1989, Cold Spring Harbor Laboratory Press:  
5 ISBN 0-87969-309-6) and subsequently hybridized for  
10 hours by adding the radiolabelled probe.

6. Construction of a plant transformation vector with  
AATP1 in antisense orientation

10 To construct a vector for the transformation of plants,  
a 1 265 bp BamHI/NdeI fragment in which the NdeI  
cleavage site is made blunt-ended with T4 polymerase is  
ligated from the coding region of the *S. tuberosum*  
AATP1 cDNA (description of the potato AATP1 cloning in  
15 Tjaden et al., 1998, The Plant Journal 16: 531-540)  
into an SmaI- and BamHI-cut vector pBinAR (Höfgen and  
Willmitzer, Plant Science 66 (1990), 221-230). The NdeI  
cleavage site is located in the AATP1 cDNA, and the  
BamHI cleavage site is derived from vector pTM1 (Tjaden  
20 et al., 1998, The Plant Journal 16: 531-540). Insertion  
of the cDNA fragment gives rise to a gene construct  
comprising the cauliflower mosaic virus 35S promoter  
(540 bp) and a 1 265 bp region of an ADP/ATP trans-  
locator 1 from *S. tuberosum* (AATP1 S.t.) in antisense  
25 orientation. The fragment was fused with the 35S  
promoter in pBinAR. The polyadenylation signal of the  
Agrobacterium tumefaciens octopine synthase gene  
(215 bp) follows in the 3' direction of the inserted  
AATP1 fragment.  
30 The overall size of the plasmid pBIN AR-AS-AATP1  
(Fig. 4) is approx. 13.3 kb.

7. Introduction of the plasmid pBINAR-ASAATP1 into the  
genome of potato plants

35 The plasmid is transferred similarly to the procedure  
described under item 5.

As the result of the transformation, transgenic potato  
plants showed a reduced level of the mRNA of a plastid

ADP/ATP translocator. This is detected by Northern blot analysis. To this end, RNA is isolated from potato leaf and tuber tissue following standard protocols. 50 µg of RNA were separated on an agarose gel (1.5% agarose, 1 × 5 MEN buffer, 16.6% formaldehyde). Following electrophoresis, the RNA was transferred to a Hybond N nylon membrane (Amersham, UK) by capillary blotting, using 20 × SSC. The RNA is immobilized on the membrane by UV irradiation. The membrane is prehybridized for 2 hours 10 in phosphate hybridization buffer (Sambrook et al., loc. cit.) and subsequently hybridized for 10 hours by adding the radiolabelled probe.

#### 8. Amino acid analysis

15 The amine acids (with the exception of proline) were measured in ethanolic extracts following HPLC separation (method of Geigenberger et al., 1996, Plant Cell & Environ. 19: 43-55).

##### 8.1 Preparation of the ethanolic extract

In each case two potato discs (total fresh weight approx. 0.2 g) are extracted for 30 minutes at 80°C in two successive steps using in each case 7 ml of 80% (v/v) ethanol and 7 ml of 50% ethanol. The total 25 extract (approximate volume 14 ml) is used for the amino acid analysis.

##### 8.2 Determination of the amino acid contents by HPLC

30 The amino acids were detected fluorometrically following pre-column derivatization of the primary amino group with o-phthaldialdehyde (OPA). To this end, an injector (Autosampler 465, Kontron, Eching) injects 35 µl of OPA reagent composed of a mixture of 5% (w/v) OPA in methanol, 0.8 M borate buffer (pH 10.4 with KOH) 35 and 3-mercaptopropionic acid (10:90:1, v:v:v) at 4°C into 35 µl of extract. After 108 seconds, 20 µl of the derivatized sample were injected.

Mobile phase A is a mixture of 1 000 ml of 12 mM sodium

phosphate (pH 6.8) and 1.6 ml of tetrahydrofuran. Mobile phase B is composed of a mixture of 250 ml of 12 mM sodium phosphate (pH 6.8), 175 ml of methanol and 110 ml of acetonitrile. The separation conditions are 5 as follows: minute 0-2, isocratic phase with 0% B, minute 2-11, linear gradient from 0 to 10% B, minute 11-17, 10% B, minute 17-27, linear gradient from 10 to 50% B, minute 27-38, linear gradient from 50 to 60% B, minute 38-44, linear gradient from 60 to 100% B, 10 minute 44-46, 100% B, minute 46-48, 100% to 0% B, minute 48-60, 0% B. A Hypersil ODS column (particle size 3  $\mu$ m, length 150 mm, diameter 4.6 mm, Knauer GmbH, Berlin) is used for the separation. The signals detected by the fluorimeter (SFM25, Kontron, Eching) 15 (excitation wavelength = 330 nm, emission wavelength = 450 nm) are integrated and evaluated by the data processing system 450-MT (Kontron, Eching).

### 8.3 Determination of the proline content

20 The proline content is determined by the method of Bates et al., 1973, Plant Soil 39: 205-207. 500  $\mu$ l of a mixture of 2 parts of 6 M  $H_3PO_4$  and 3 parts of 75% acetic acid and 500  $\mu$ l of ninhydrin solution (600 mg per 20 ml of 75% acetic acid) are added to 25 200  $\mu$ l of extract. After incubation for 45 minutes at 95-100°C, the test mixture is placed on ice and mixed with 300  $\mu$ l of toluene. Following phase separation, the top phase is transferred into a microcuvette, and the OD is measured at 515 nm. The proline content is 30 determined by comparison with a calibrating plot (1-50  $\mu$ M proline).

5 Tab. 1: Overview of the amino acid content in the Solanum tuberosum wild type and in the transformed potato plants comprising the ATP/ADP translocator gene in sense orientation (Sense-98 and Sense-62) or in antisense orientation (Antis-594 and Antis-595).

<b>Genotype</b>	Aspartate	Glutamate	<b>Aspartic acid</b>	Serine	Glutamine
Wild type	2.020	2.090	11.190	1.066	5.586
Sense-98	1.656	2.238	8.986	1.008	7.409
Sense-62	1.924	1.540	12.533	0.838	6.949
Antis-594	0.746	4.123	1.256	0.875	5.633
Antis-595	0.880	4.670	4.344	1.057	6.931
<b>Genotype</b>	Tyrosine	Valine	<b>Methionine</b>	Tryptophan	Phenylalanine
Wild type	1.201	3.589	0.986	0.519	1.544
Sense-98	1.840	4.010	1.098	0.780	2.286
Sense-62	1.440	3.633	0.920	0.506	1.620
Antis-594	0.474	2.620	0.403	0.143	2.039
Antis-595	0.228	2.340	0.510	0.019	1.716
<b>Genotype</b>	Glycine	<b>Threonine</b>	Histidine	Alanine	Arginine
Wild type	0.473	1.168	0.699	1.036	1.809
Sense-98	0.507	1.318	0.865	1.694	2.122
Sense-62	0.442	1.197	0.838	1.165	2.008
Antis-594	0.448	0.612	0.265	1.824	0.493
Antis-595	0.641	0.574	0.292	1.562	0.396
<b>Genotype</b>	<b>Isoleucine</b>	Leucine	<b>Lysine</b>	Proline	Free ASS, total
Wild type	1.450	0.212	1.027	0.595	41.7
Sense-98	1.819	0.296	1.310	0.552	44.7
Sense-62	1.445	0.195	1.291	0.546	43.9
Antis-594	0.681	0.142	0.270	0.451	27.7
Antis-595	0.535	0.124	0.228	0.470	33.0

All data in  $\mu\text{mol/gFW}^{-1}$

**Patent Claims:**

1. Transformed plant and its progeny, characterized in that its regulatory sequences and/or gene copy number of an ATP/ADP translator gene are modified in such a way that it exhibits one or more amino acids simultaneously in modified amounts in comparison with a corresponding untransformed plant.
- 10 2. Transformed plant and its progeny according to Claim 1, characterized in that it exhibits an increased transport capacity for ATP into the chloroplast membrane.
- 15 3. Transformed plant and its progeny according to Claim 1 or 2, characterized in that it exhibits predominantly one or more essential amino acid(s) in modified amounts.
- 20 4. Transformed plant and its progeny according to one of Claims 1 to 3, characterized in that it exhibits one or more essential amino acid(s) whose content is increased over that of the untransformed plant.
- 25 5. Transformed plant and its progeny according to one of Claims 1 to 4, characterized in that it is a useful plant.
- 30 6. ATP/ADP translocator gene for use in a plant according to one of Claims 1 to 5 with an *Arabidopsis thaliana* nucleotide sequence (EMBL Accession No. Z49227) encoding the amino acid sequence shown in Fig. 1.
- 35 7. ATP/ADP translocator gene according to Claim 6 with a naturally, chemically synthesized, modi-

fied, artificially generated nucleotide sequence with essentially the same action or with heterologous nucleotide sequences encoding an ATP/ADP translocator or allelic variations or isoforms thereof or with mixtures thereof.

5 8. ATP/ADP translocator gene according to Claim 6 or 7 with operably linked regulatory nucleotide sequences.

10 9. ATP/ADP translocator gene according to one of Claims 6 to 8 with an upstream, operably linked promoter.

15 10. Gene structure comprising an ATP/ADP translocator gene according to one of Claims 6 to 9 and regulatory sequences linked operably to this gene.

20 11. Vector comprising an ATP/ADP translocator gene according to one of Claims 6 to 9 or a gene structure according to Claim 10.

25 12. Vector according to Claim 11 comprising additional regulatory nucleotide sequences, preferably from the group of the promoters, terminators or translation enhancers, and nucleotide sequences for the replication in a suitable host cell or for integration into its genome.

30 13. Seeds of the plant according to one of Claims 1 to 5.

14. Tissue or cells or material capable of propagation from the plant according to one of Claims 1 to 5.

35 15. Method of generating a plant with an increased amino acid content according to one of Claims 1 to 5, characterized in that an ATP/ADP translocator gene according to one of Claims 6 to 9 or a gene

structure according to Claim 10 or a vector according to Claim 11 or 12 is transferred by recombinant methods.

5 16. Use of the transformed plant according to one of Claims 1 to 5 as useful plant or fodder plant.

10 17. Use of the transformed plants according to one of Claims 1 to 5 or of tissue or cells thereof or of extracts thereof in sectors of agriculture, the feedstuff industry, the pharmaceutical industry or in the health sector.

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Veröffentlicht:

— Mit internationalem Recherchenbericht.

Zur Erklärung der Zweibuchstaben-Codes, und der anderen  
Abkürzungen wird auf die Erklärungen ("Guidance Notes on  
Codes and Abbreviations") am Anfang jeder regulären Ausgabe  
der PCT-Gazette verwiesen.

(54) Title: PLANTS HAVING ALTERED AMINO ACID CONTENTS AND METHOD FOR THE PRODUCTION THEREOF

**A1** (54) Bezeichnung: PFLANZEN MIT VERÄNDERTEM AMINOSÄUREGEHALT UND VERFAHREN ZU DEREN HERSTELLUNG

(57) Abstract: The invention relates to transformed plants and their descendants, which have altered regulatory sequences and/or number of gene copies of the ATP/ADP-translocator-gene in such a manner that they have one or more amino acids simultaneously in altered amounts in comparison with a non-transformed plants. The invention also relates to a method for the production of said plants in addition to their use as crop plants or in the animal feedstuffs industry.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft transformierte Pflanzen und deren Nachkommen, die in den regulativen Sequenzen und/oder der Genkopienzahl des ATP/ADP-Translokator-Gens derart verändert sind, dass sie gegenüber einer nicht transformierten Pflanze eine bis mehrere Aminosäuren gleichzeitig in veränderten Mengen aufweisen. Ferner betrifft die vorliegende Erfindung ein Verfahren zur Herstellung dieser Pflanzen sowie deren Verwendung als Nutzpflanze oder in Bereichen der Futtermittelindustrie.

**WO 01/20009 A1**

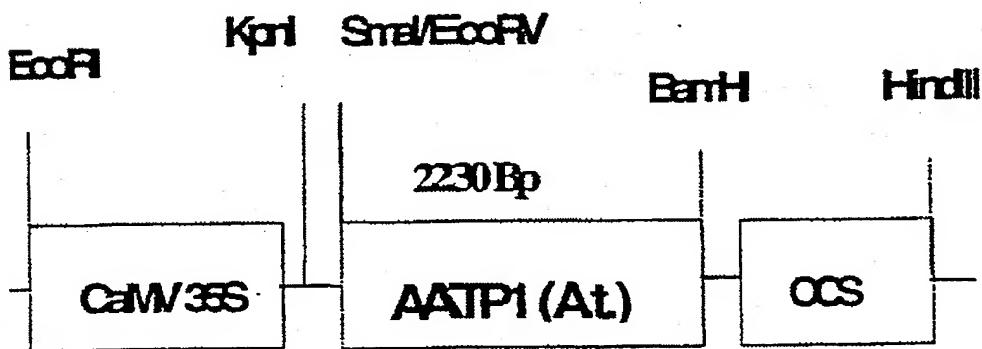
Fig. 1: *Arabidopsis thaliana* cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Z49227)

Fig. 2: *Solanum tuberosum* cDNA corresponding to the coding region of the chloroplast ATP/ADP translocator 1 (EMBL Accession Number Y10821).

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Fig. 3: Plant transformation vector pBIN-AR-AATP1 for expressing the ATP/ADP translocator in sense orientation



CaMV 35S: cauliflower mosaic virus 35S promoter

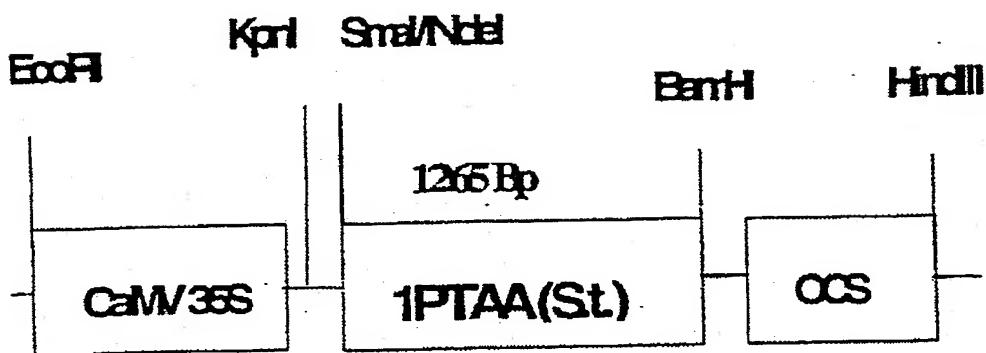
AATP1 (A.t.): EcoRV/BamHI fragment of the *Arabidopsis thaliana* ATP/ADP translocator 1 in sense orientation

OCS: polyadenylation signal of the *Agrobacterium tumefaciens* octopine synthase gene

Those restriction sites which cut the vector only once are also shown.

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Fig. 4: Plant transformation vector pBIN-AR-AATP1-AS for expressing the ATP/ADP translocator in antisense orientation



CaMV 35S: cauliflower mosaic virus 35S promoter

1PTAA (S.t.): BamHI/NdeI fragment of the Solanum tuberosum ATP/ADP translocator gene in antisense orientation

OCS: polyadenylation signal of the Agrobacterium tumefaciens octopine synthase gene

Those restriction sites which cut the vector only once are also shown.

U.S. PATENT AND TRADEMARK OFFICE

# Declaration, Power of Attorney and Petition

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0093/000043

We (I), the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

the specification of which

is attached hereto.

was filed on \_\_\_\_\_ as

Application Serial No. \_\_\_\_\_

and amended on \_\_\_\_\_

was filed as PCT international application

Number PCT/EP/00/07625 \_\_\_\_\_

on 05 August 2000 \_\_\_\_\_

and was amended under PCT Article 19

on \_\_\_\_\_ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
19944212.6	Germany	15 September 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

We (I) hereby claim the benefit under Title 35, United States Codes, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
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(Application Number)	(Filing Date)
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We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)

And we (I) hereby appoint Nixon & Vanderhye P.C., Attorneys at Law, 1100 North Glebe Road, Arlington, Virginia 22201-4714, our attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to sign the drawings, to receive the patent, and to transact all business in the Patent Office connected therewith.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Declaration

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0093/000043

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Rec'd PCT/PTO 13 SEP 2002

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